Greater yogurt consumption is associated with increased bone mineral density and physical function in older adults

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--Manuscript Draft--

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Abstract:	Summary In this cohort of community dwelling older adults (>60 yrs), we observed significant positive associations between the frequency of yogurt intake and bone health. Introduction The associations of yogurt intakes with bone health and frailty in older adults are not well documented. The aim was to investigate the association of yogurt intakes with bone mineral density [BMD)], bone biomarkers and physical function in 4,310 Irish adults from the Trinity, Ulster, Department of Agriculture aging cohort study (TUDA). Methods Bone measures included total hip, femoral neck and vertebral BMD with bone biochemical markers. Physical function measures included Timed Up and Go (TUG), Instrumental Activities of Daily Living Scale and Physical Self-Maintenance Scale. Results Total hip and femoral neck BMD in females were 3.1 - 3.9 % higher among those with the highest yogurt intakes (n= 970) compared to the lowest (n= 1,109; P <0.05) as were the TUG scores (-6.7%; P = 0.020). In males, tartrate-resistant acid phosphatase (TRAP 5b) concentrations were significantly lower in those with the highest yogurt intakes (-9.5%; P <0.0001). In females, yogurt intake was a significant positive predictor of BMD at all regions. Each unit increase in yogurt intake in females was associated with a 29% lower risk of osteopenia (OR 0.71; 95% CI 0.51 - 1.01; P=0.037) and a 37% lower risk of osteoporosis (OR 0.63; 95% CI 0.44 - 0.91; P=0.014) and in males, a 51% lower risk of osteoporosis (OR 0.49; 95% CI 0.25 - 0.94; P=0.032). Conclusion In this cohort, higher yogurt intake was associated with increased BMD and physical function scores. These results suggest that improving yogurt intakes could be a valuable and cost-effective health strategy for maintaining bone health in older adults.
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Author Comments:	Drs. J. Kanis; F. Cosman Editors-in Chief Osteoporosis International 27th August 2016 Dear Co-Editors, We would be very grateful if you would consider our manuscript "Greater yogurt consumption is associated with increased bone mineral density (BMD) and physical function in older adults" for publication in Osteoporosis International Osteoporosis is an increasingly common, chronic condition estimated to affect over 200 million individuals worldwide. Maintaining an optimal nutritional status is a key preventative measure, particularly for older adults (>60 years). Of the major food groups, dairy foods (including milk, yogurt and cheese) are one of the richest sources of the macro and micro nutrients that contribute to bone health. There is however, little information on the associations of yogurt intake, with BMD and bone health bio-markers in addition to concomitant measurements of physical function in this older population. In this study we investigate the associations of daily dairy intakes (in particular yogurt) and the associations with BMD, bone biomarkers and measures of physical function in 4,310 older adults (60-102 years). The participants were originally recruited to the Trinity Ulster Department of Agriculture (TUDA) aging cohort study, a large study of older Community dwelling Irish adults designed to investigate nutritional factors and gene-nutrient interactions in the development of chronic diseases of aging. Data for the current study relate to >4,000 participants, making it the largest study to-date examining yogurt intakes and associations with bone health and frailty. Bone biomarkers included serum osteocalcin, Bone specific alkaline phosphatase, C-terminal telopeptides of type I collagen and Tartrate-resistant acid phosphatase (TRAP 5b). BMD was measured at the total hip, femoral neck and vertebral spine. Physical function measures included Timed get Up and Go and the Instrumental Activities of Daily living scale. Total hip and femoral BMD in women were 3.1 - 3.9 % higher amongst those with the highest yogurt intakes, as were the TUG scores (-6.7%). TRAP 5b concentrations were significantly lower in men with the highest yogurt intakes (-9.5%). In a regression model examining predictors of osteoporosis, each unit increase in daily yogurt intake was associated with a 29% lower risk of osteopenia and a 37% lower risk of osteoporosis in women (what about osteopenia in men) and a 51% lower risk of osteoporosis in men Our findings provide evidence that an increase in the frequency of yogurt intake is positively associated bone health status and measurements of physical function in a cohort of older adults. We believe the findings of the current study will contribute significantly to the growing evidence supporting a beneficial role for yogurt in health. Thank you for taking the time to consider this manuscript. Yours sincerely, Dr Eamon Laird

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Response to Reviewers:	Reviewer 1 This is a well written observational cross-sectional study carried out in a large cohort of community dwelling older (>60 years) females and male subjects. Response: We thank reviewer 1 for their helpful comments regarding the manuscript. The clarifications suggested by the reviewer are welcomed and the specific replies to each comment are shown below. 1. My main concern is the excess of reliance on statistical analysis without considering the pathophysiological relevance of the significance as computed by using various tests including hierachical regression models with adjustement for about 15 variables. For instance, what is the biological meaning of reporting that an increase in serum 25OHD is associated with greater (Odd ratio: 1.0, 95%CI 1.00-1.02 !) risk of osteoporosis with a probability level of <0.0001 (Table 5) ? This kind of significance test amalgamates precision with effect size, thus muddling two essential aspects of data interpretation. A weak association between an extremely mild increase in serum 25OHD and the risk of osteoporosis is incorrectly interpreted as “important“ because it is statistically significant. In order to control Type I error rate (i.e. to conclude there is a difference though none exists) when multiple hypothesis are tested, a correction has to be applied. Among several statistical correction tools, the Bonferroni correction is often used, making it more difficult to reject the null hypothesis. Response: We agree with the reviewer that this is an unexpected result and that we should have mentioned this in the text (we did not discuss this in the results or discussion). We feel this unexpected result could be due to the fact we did not include those on vitamin D supplements as a variable in the multinomial regression model. We have now included this for both women and men (Tables 5 and 6). Serum 25(OH)D is no longer a significant predictor of bone health for women or men. However, yes to vitamin D supplements is now a significant predictor of bone health. For women, those on vitamin D supplements had a significantly reduced risk of osteopenia (OR 0.51; 95% CI 0.34 – 0.76; P=0.001) and a significantly reduced risk of osteoporosis (OR 0.41; 95% CI 0.26 – 0.64; P<0.0001). For men, those on vitamin D supplements also had a significantly reduced risk of osteoporosis (OR 0.40; 95% CI 0.22 – 0.72; P=0.003). These results have been added both to Tables 5 and 6. With the addition of the vitamin D supplement variable to the model, the positive effect of daily yogurt intakes on reducing the risk of impaired bone health has also increased (lines 246-248). In regards to the Bonferroni correction, the authors feel that it is statistically inappropriate for the multinomial regression model as each variable included is controlling for the other. However, for the other analysis (Tables 2-3 and Supplemental Tables 2-3) we have used the Bonferroni correction. We apologise that this was not made clear in the statistical methods and we have now added a sentence to this effect and a footnote to each of the appropriate Tables. It is important to note however, that in doing the Bonferroni correction, the correction directly targets the Type 1 error problem, but it does so at the expense of Type 2 error. By changing the p value needed to reject the null (or equivalently widening the uncertainty intervals) the number of claims of rejected null hypotheses will indeed decrease on average. Although this reduces the number of false rejections, it also increases the number of instances that the null is not rejected when in fact it should have been. Thus, the Bonferroni correction can severely reduce our power to detect an important effect (Gelman et al 2012). 2. According to DXA-BMD measurements (presumably by using either Total Hip or Femoral Neck BMD? This referenced skeletal site has to be clearly indicated), odd ratios were calculated for 15 variables in each gender (Tables 5 and 6). In females, 3 and 5 variables were found to be significantly associated with the risk of osteopenia (+Age, -BMI, -Daily Yogurt Serving) and osteoporosis (+Age, -BMI, +25OHD, -Daily Yogurt Serving), respectively. In males, 3 variables were found to be significantly associated with the risk of osteopenia (-BMI, +Daily Cheese Serving, -Daily Meat Serving) and osteoporosis (-BMI, -Daily Yogurt Serving, +Daily Cheese), respectively. Some of these associations appear to be significant statistically, but without any biological significance. Thus, in females mean serum 25OHD varies very little among the 3 groups categorized according to the frequency of yogurt intakes (Table 2). Similarly minimal variations of serum 25OHD were recorded in females distributed

according to the frequency of milk (Supplemental Table 2) and cheese (Supplemental Table 4) intakes.

Response: We have now inserted a sentence explaining the referenced skeletal sites for Tables 5 and 6 as a footnote on each. In response to the results of the multinomial regression model, some of the results that were observed were expected and have been reported extensively in the literature (e.g. increasing age, and lower BMI associated with increased risk of osteopenia/osteoporosis). We feel it is interesting some of the dietary components were also significant as few studies often examine these as predictors of bone health and thus little is known on the effects of diet on bone health. As stated in response to query 1, the multinomial regression model is a robust statistical model with each variable acting as a control/adjustment for another. In regards to Table 2, supplemental Tables 2 and 4 it is actually a positive that 25(OH)D concentrations did not differ between the groups as it can then be ruled out as a potential likely driver of any effects on bone health that we observed.
3. To determine the predictors of bone fragility (osteopenia and osteoporosis) a multinomial logistic regression model was used (with normal bone health as the reference category) with relevant co-predictors including serum 25OHD as a nominal variable (P.8, lines 184-190). As presented in Table 5, in females the risk of osteoporosis would be positively increased by 1.01 (95% CI 1.00-1.02, P<0.0001) by an augmentation of serum 25OHD. This is an example of highly statistical significance without any pathophysiological significance. This kind of statistic test mixes effect size and precision, thus muddling two essential aspects of data interpretation. Confidence intervals provide both an estimate of the effect size and the precision of the measurement.

Response: We have amended these results as explained in the response to queries 1, 2 and 6.

4. P.7, lines 168-169. References should be given for the functionality measures by PSM and IADL.

Response: A reference has now been inserted (line 176).

5. P.8, line183-184. BMD unit is in gram of hydroxyapatite equivalent divided by the DXA-scanned surface (g/cm2) of the region of interest. Therefore it does not correspond to a “concentration“. This wrong unit designation should be corrected.

Response: We have now removed any mention of ‘concentration’ to BMD measures or results in the manuscript.

6. Table 5. Females statistical analysis. Increasing age significantly augments the Odd ratio (augments the risk) for both osteopenia (1.03 |1.00-1.06| P=0.043) and osteoporosis (1.04 |1.01-1.08| P=0.008) (Table 5). Increasing BMI (0.92 |1.00-1.06| P<0.0001). Physical activity and Yogurt consumption reduces the Odd ratio (reduces the risk) of osteoporosis (Table 5). These findings are consistent with previous knowledge on the risk factors of bone fragility in later age. A discordant finding concerns bone fragility (osteopenia, osteoporosis) related to the vitamin D status. It is reported that an increase in serum 25OHD rises the risk of osteoporosis (Table 5). This is inconsistent with most published studies providing evidence that increasing vitamin D status, as assessed by measuring the serum level of 25OHD, reduces bone turnover in menopausal women and is associated with a lower risk of fragility fractures with aging.

Response: We have agree with the reviewer and have amended these results as explained in the response to queries 1, 2 and 3.

7. -Table 6. Males statistical analysis. Increased BMI significantly reduces the Odd ratio for osteoporosis. Increase in both yogurt and meat consumption also reduces the risk of osteopenia and osteoporosis. These findings corroborate several published results. In contrast, in this epidemiological study an increased consumption of cheese would augment the risk of developing either osteopenia or osteoporosis. The authors recognize that this statistical finding is in contradiction with recent reports indicating that an increase of cheese consumption exerts a rather bone-protective effect. They

mention the hypothesis that a high sodium content of certain varieties of cheese would increase the urinary excretion of calcium and, thereby, would negatively influence bone mineral balance. However, the sodium-induced calciuria hypothesis is not supported by any long term observations. To mention an untenable mechanistic hypothesis to account for a statistically unexpected and contradictable result lowers the scientific quality of the discussion.

Response: We acknowledge the reviewers comments the sodium-induced calciuria hypothesis is not supported by any long term observations (e.g. the recent Women's Health initiative Study) and we have mentioned this in the text. However we also recognize that this could offer a potential hypothesis to explain our observed results and given that it has not been extensively examined in the literature, in particular for the effects on men, we feel we must mention it as a possibility.

8. P.8, lines 195 - P9, line 196. The sentence "The majority of participants were females, who were significantly older, lighter, and contained a higher proportion of individuals receiving bone, vitamin D or calcium supplements in comparison with males." is not clear. The word "bone" should be deleted.

Response: We have deleted this from the sentence.

9. P.9, lines 220 – P10, lines 221-223. The differences highlighted in terms of milk consumption, for Time up and Go, IADL and PSM (Supplemental Table 2 and 3, not Suppl.Tables 1 and 2) are, though statistically significant, of trivial biological significance. A more stringent statistical analysis using a multple-comparison procedure (e.g. Bonferroni's method) that increases the critical F or t is needed for declaring the comparison to be significant.

Response: As stated in response to query 1, these results are adjusted for Bonferroni. We again apologize for not making this clear in the manuscript and have added this as a footnote to the Tables of interest.

10. P.10, line 224. Please substitute Supplemental Table 3 and 4 by Supplemental Table 4 and 5.

Response: We have now switched these Supplemental Tables.

11. -Table 2 and Table 3. Reference range of the listed biochemical markers (CTX, OC, BAP; TRAP 5b, 25OHD and PTH) should be indicated in the legend to these two tables.

Response: We have now added these reference ranges in the methods section and in the legends to Table 2 and 3.

12. P.10, lines 244-245 and P.11, lines 246-247. In men, Table 6 does not corroborate the statement that in men increased yogurt consumption was associated with a significant decrease in the Odd ratio of osteopenia (Table 6, 0.85, 95% CI 0.58-1.24, P =0.410).

Response: We apologize for this error and have now amended the sentence.

13. P.11, lines 256-259. The reference #22 (Hochberg et al., 2002 J Clin Endocrinol Metab) is a meta-analysis of randomized placebo-controlled trials in postmenopausal women who were all diagnosed at baseline as being osteoporotic. The effects of antiresorptive pharmaceutical agents (particularly the bisphosphonates alendronate and risedronate) were analyzed regarding the later incidence of nonvertebral fractures in relation with early changes in BMD and biochemical markers (BCM). This important meta-analysis estimated that a 3% increase in hip BMD reduced nonvertebral fracture risk by about 46%, and a 70% decrease in resorption BCM reduced nonvertebral fracture risk by about 40 %. In contrast, the submitted observational cross-sectional study was carried out in less than 60 % of osteoporotic women (360/626=57.5%, Table 5). Therefore, the hypothesis that the effects of increased yogurt consumption on BMD and resorption biochemical markers would cause in the long term such a large reduction in nonvertebral fracture risk should be expressed with some caution.

Response: We thanks the reviewer for this comment and have inserted this information into the text and we have also stated that such a reduction is expressed with caution ‘However it is important to note that the meta-analysis [27] was conducted in women all diagnosed with osteoporosis whereas in the current study only 60% of the women were osteoporotic and thus the potential for yogurt to reduce fractures at the same rate should be viewed with caution’ (lines 273-276).

14. The punctuation should be checked throughout the manuscript, particularly some full stops are missing e.g.: P13, line 302.

Response: We apologize for this error and have now checked and amended the manuscript throughout.

References

Gelman, Andrew, Jennifer Hill, and Masanao Yajima. "Why we (usually) don't have to worry about multiple comparisons." Journal of Research on Educational Effectiveness 5.2 (2012): 189-211.

Reviewer 2:
In this cross-sectional study conducted in a large cohort of healthy men and women aged 60 years and older, an association between bone health and physical function, and yogurt consumption was assessed. The results indicate some positive association, and suggest the possibility of yogurt based preventive regimens for maintaining bone health. This paper raises the following comments.

Response: We thank reviewer 2 for their valuable comments regarding the manuscript. The clarifications suggested by the reviewer are again highly appreciated and the specific replies to each comment are shown below.
Major points:

1. The level of physical activity in yogurt consumers and non-consumers should be presented.

Response: We have now added the sentence ‘In yogurt consumers, the proportion who answered yes to physical activity was 80.9% while in non-consumers it was 74.7%.’ (lines 207-208).

2. The size of yogurt servings as well as whether yogurts are usually enriched in milk powder in the region should be specified, in other words, whether 120 g of yogurt are providing more calcium and protein than 120 g of milk.

Response: We have no data in relation to the serving size of each dairy type or the particular product brand (lines 136 - 137) and thus we cannot state whether there is a difference in the calcium or protein content. We have mentioned this as a limitation of the study (lines 355-358). However, we have also mentioned the average serving intake size of milk, yogurt and cheese in a representative study of older Irish adults (>65 yrs) recruited at the same time period (as part of the National Adult Nutrition Study (NANs) (lines 137 - 140).

3. The results should also be adjusted for calcium and protein of dairy origin, to address the issue of yogurt specificity.

Response: As stated in our response to major point 2, we have no data in relation to the serving size of each dairy type or the particular product brand and thus we cannot adjust for the exact protein or calcium amount of dairy origin. However, through all of the analysis of the associations of yogurt with BMD and bone health we have adjusted for the frequency of intakes for other dairy products such as milk and cheese and have adjusted for the frequency of intakes for non-dairy products including red and white meat (total meat), oily and white fish (total fish) and egg intakes.

4. What would be the results when adjusted for weight and height, instead of BMI?

Response: We have repeated the analysis with adjustment for weight and height instead of BMI and the results are very similar to what has been reported in the manuscript. For women, total hip BMD was 3.1% higher (P=0.004) and the femoral neck BMD was 3.9% higher (P<0.0001) in those with the highest yogurt intakes compared to the lowest. All other results for women were also similar. For men, the concentrations of the bone biomarker TRAP were 9.6% lower in those with the highest yogurt intakes compared to the lowest (P=0.004).

5. The associations differ between men and women. What kind of explanation can be provided?

Response: The results differ significantly between genders and we suggested that given significantly fewer men than women were high yogurt consumers, it is possible that there were only subtle bone turnover changes in men as not enough yogurt was being consumed to affect BMD but enough to affect bone turnover (lines 283 - 286). However, it is beyond the scope of the current study to detect the reason behind the gender differences.

5. Did T-score differ between groups as well, without or with adjustment?

Response: For women only (without adjustment), total hip T-scores were significantly higher (P=0.006) as were femoral neck T-scores (P<0.0001) in those with the highest yogurt intakes compared to the lowest/none yogurt intake group. For women only (with adjustment), total hip T-scores were significantly higher (P=0.004) as were femoral neck T-scores (P<0.0001) in those with the highest yogurt intakes compared to the lowest/none yogurt intake group. There was no difference in vertebral T-scores with or without adjustment.
For men only, vertebral T-scores were significantly higher in the low yogurt consumer group compared with the lowest/none yogurt intake group with adjustment (P =0.028) and without adjustment (P=0.040). There was no differences in total hip or femoral neck T-scores with or without adjustment.

Minor points

1. Abstract: cost-effectiveness of yogurt consumption has not been evaluated nor discussed in the paper.

Response: This statement has been removed from the abstract

2. Introduction: the paper showing reduced fracture risk and mortality with fermented dairy products may be quoted.

Response: This reference has now also been mentioned in the introduction (lines 92 - 93).

3. Did the subjects without BMD measurements differ from those included in the present study?

Response: The TUDA study was based on three diseased cohorts: hypertension, cognitive dysfunction and osteoporosis. Those who were on current bone treatment were removed from the analysis. Subjects in the cognitive cohort (this cohort did not have BMD measures) were recruited from geriatric clinics and a day hospital service and cognitive impairment was assessed based on testing with the RBANS (Repeatable Battery for the Assessment of Neuropsychological Status). This cohort was significantly older and their ability to accurately recall dietary intakes may be compromised.

4. Which database was used to calculate T-score in men? The same as for women?

Response: The database used was from NHANES III for the femoral neck and total hip and the manufacturer's own database for the vertebral.

	5. Prevalent fracture should be reported Response: A very small proportion of the cohort self-reported either a hip fracture (n = 54) or self-reported a vertebral fracture (n = 128) and thus we could not examine the associations with dairy intakes as the study was not powered to detect or examine prevalent fracture rates. Additionally, we cannot ensure the accuracy of these fractures rates as they have been self-reported. 6. Phosphate supplements are very unusual. What was the reason for these 6%? Response: We agree with the reviewer that phosphate supplements on their own is quite unusual. However, the TUDA study collected information on all supplements/medications consumed and thus an exhaustive search was conducted for any preparation, medication or supplement that contained any phosphate to be as thorough as possible. 7. P 11: the paper by Hochberg et al cannot be used in the present discussion since the changes in BMD were achieved with pharmacological agents. There is no argument that the relationship may be similar with nutritional intervention. Response: We acknowledge the reviewers comments and although the changes in BMD were achieved with pharmacological agents in the paper by Hochberg et al., there is still a possibility that there is a similar relationship with nutritional intervention (dairy products) as this has not been investigated before. However we have modified this part of the discussion in line with suggestions with reviewer 1 and have stated that these possibilities should be treated with caution until further evidence is available. 8. The possibility that yogurt consumption may be a marker of a healthy lifestyle should be discussed. Response: We agree with the reviewer that yogurt consumption may be a marker of a healthy lifestyle and have discussed this in the manuscript (lines 315 - 316).

Greater yogurt consumption is associated with increased bone mineral density and physical function in older adults

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Conflict of interest

Eamon Laird, Anne Molloy, Helene McNulty, Mary Ward, Kevin McCarroll, Leane Hoey, Catherine Hughes, Conal Cunningham, JJ Strain and Miriam Casey declare that they have no conflict of interest

Mini-Abstract

In this cohort of community dwelling older adults (>60 yrs), we observed significant positive associations between the frequency of yogurt intake with measures of bone density, bone biomarkers and indicators of physical function. Improving yogurt intakes could be a valuable health strategy for maintaining bone health in older adults.

Abstract

Introduction The associations of yogurt intakes with bone health and frailty in older adults are not well documented. The aim was to investigate the association of yogurt intakes with bone mineral density [BMD], bone biomarkers and physical function in 4,310 Irish adults from the Trinity, Ulster, Department of Agriculture aging cohort study (TUDA).

Methods Bone measures included total hip, femoral neck and vertebral BMD with bone biochemical markers. Physical function measures included Timed Up and Go (TUG), Instrumental Activities of Daily Living Scale and Physical Self-Maintenance Scale.

Results Total hip and femoral neck BMD in females were 3.1 - 3.9 % higher among those with the highest yogurt intakes (n= 970) compared to the lowest (n= 1,109; P <0.05) as were the TUG scores (-6.7%; P = .013). In males, tartrate-resistant acid phosphatase (TRAP 5b) concentrations were significantly lower in those with the highest yogurt intakes (-9.5%; P <0.0001). In females, yogurt intake was a significant positive predictor of BMD at all regions. Each unit increase in yogurt intake in females was associated with a 31% lower risk of osteopenia (OR 0.69; 95% CI 0.49 – 0.96; P=0.032) and a 39% lower risk of osteoporosis (OR 0.61; 95% CI 0.42 - 0.89; P=0.012) and in males, a 52% lower risk of osteoporosis (OR 0.48; 95% CI 0.24 - 0.96; P=0.038).

Conclusion In this cohort, higher yogurt intake was associated with increased BMD and physical function scores. These results suggest that improving yogurt intakes could be a valuable public health strategy for maintaining bone health in older adults.

Introduction

Osteoporosis is an increasingly common, chronic condition estimated to affect over 200 million individuals worldwide [1] with 6% of men and 21% of women aged 50-84yrs affected in the EU alone [2]. It is characterized by decreased bone mineral density (BMD) with a significantly increased risk of fracture and subsequently, morbidity and mortality [3]. The condition has been estimated to cause over 8.9 million fractures annually, with osteoporotic fractures accounting for 0.8% of the global burden of non-communicable disease and the loss of over 5.8 million disability-adjusted life years (DALYs) [4-6].

The development of osteoporosis can be influenced by a range of both demographic and lifestyle factors [1,7,8]. However, maintaining an optimal nutritional status is also a key preventative measure, particularly for older adults (>50 years) [9]. Of the major food groups, dairy foods are one of the richest sources of the macro and micro nutrients that contribute to bone health such as protein, calcium, magnesium and the B-vitamins [10-14]. For example, dairy products are the primary source of calcium across most industrialized countries in Europe and the USA [11,12]. Previous data from observational studies and randomized controlled trials (RCTs) have reported significant positive associations between dairy intakes and bone health as reviewed in recent commentaries and Government reports [15,16]. In one 12-yr follow-up analysis of the Framingham Offspring Study (*n*= 2,506; mean age 55 yrs), yogurt intake alone was positively associated with hip trochanter BMD and had a weak protective trend with hip fracture reduction [17]. Furthermore, fermented milk products have been associated with a lower fracture incidence and mortality [18]. There is however, little information on the associations of yogurt intake with bone health bio-markers and with measures of functionality. In the current study, we examined the association of yogurt intakes with BMD, biochemical

markers of bone health and physical function measures in a large cohort of free-living older adults (*n*= 4,310, age range 60-102 yrs).

Subjects and Methods

Data analyzed for the current study originated from the Trinity Ulster Department of Agriculture (TUDA) ageing cohort study, a large study of older Irish adults (>60 yrs) designed to investigate nutritional factors, related gene-nutrient interactions and a range of health and lifestyle factors in the development of chronic diseases of aging. Further details of sampling and recruitment have been described previously [19-22]. Of the 5,186 participants recruited, 3 with severe frailty (replied no or had a missing answer to the self-feeding question in the Physical Self-Maintenance questionnaire (PSM)), 866 with cognitive impairment (Mini-Mental State Examination (MMSE) score <25) and those with a missing response to the yogurt intake question (*n*= 7) were excluded from the physical function analysis leaving a total of 4,310 participants (Supplemental Figure 1). Approximately 1,699 participants did not have BMD measures taken. In addition to these exclusions, participants who reported receiving medications that could affect bone mineral metabolism (Bisphosphonates; Aromatase Inhibitors; Gonadotropin releasing hormone analogues or Luteinizing hormone releasing agonists; Anti-androgen medication; Parathyroid hormone (PTH) treatment; Strontium treatment; Anti-epileptic medications; Paget's disease treatment) were also excluded from the BMD and bone biomarker analysis (Supplemental Figure 1). Ethical approval was granted by the relevant authorities in each jurisdiction: the Research Ethics Committee of St. James's Hospital and The Adelaide and Meath Hospital, Dublin, and the Office for Research Ethics Committees Northern Ireland (ORECNI; reference 08/NI/RO3113) with corresponding approvals from the Northern and Western Health and Social Care Trusts, Northern Ireland.

Lifestyle and medications

Data associated with lifestyle factors were obtained by questionnaire. Information included gender, age, ethnicity, physical activity (reported as yes/no in the last two weeks), smoking status and alcohol intake. Full details of dietary supplement and vitamin use including dose, frequency and duration was confirmed from packaging or prescription information. A small number of supplements listed were unidentifiable or contained unidentifiable ingredients; individuals consuming such supplements were excluded from analysis.

Dietary dairy intake servings

Participants were given a modified food frequency questionnaire (FFQ) which asked if the participant consumed yogurt, milk (approximated as glasses of milk per day) and/or cheese and if yes, how often for each dairy type. The total frequency of the daily intake serving for yogurt, milk and cheese was calculated from the FFQ responses (Supplemental Table 1). These values were then separated into tertiles of non-consumers, low consumers and high consumers for each dairy type. Information was also recorded for the frequency of consumption of red meat and poultry (total meat), oily and white fish (total fish) and egg intakes. No product brand name or serving size information was available for the dairy intakes, however the average serving intake size in a representative study of older Irish adults (>65 yrs) recruited at the same time period (as part of the National Adult Nutrition Study (NANs)) was 114 grams (g) for yogurt, 123g for milk and 35g for cheese [23].

Biochemical analysis

A non-fasting blood sample (50ml) was collected by venipuncture into an evacuated clotting tube (Sarstedt; Numbrecht, Germany) by a trained phlebotomist. Samples were kept chilled and centrifuged (3000 rpm for 15 minutes) within 3 hours of collection and serum aliquots

were labeled and stored at -80°C until required for analysis. Serum bone bio-markers were measured in duplicate using an automated enzyme immunoassay method (EIA) following the manufacturer's instructions (Triturus®, Immunodiagnostics (IDS) limited, Boldon, Tyne & Wear, UK). Inter-assay CVs were as follows: Serum osteocalcin (OC) <4.5% (reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml), C-terminal telopeptides of type I collagen (CTX) <3.1% (reference range is 0.020 ng/mL to 3.380 ng/ml), Bone specific alkaline phosphatase (BAP) <1.5 % (reference range for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml) and Tartrate-resistant acid phosphatase (TRAP 5b) <1.6% (reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml). Intact PTH was measured at St. James's Hospital, Dublin using an electrochemiluminescence immunoassay (ECLIA) (Modular E170, Roche Diagnostics, Dublin, Ireland) with an inter-assay CV of <2.9% and an assay measurement range of 1.2 – 5000 pg/ml. Vitamin D (25-hydroxyvitamin D (25(OH)D)) concentrations were quantified using LC-MS/MS (API 4000; AB SCIEX; Chromsystems GmbH) with an inter-assay CV of <5.7% (detection range 7.5 – 624 nmol/L) [19,20]. Renal function tests (creatinine) were analyzed using a Roche Cobas c701 (Roche 8000 modular system) with an inter-assay CV <5%. Glomerular filtration rate (GFR) was estimated by use of the Cockcroft-Gault equation.

BMD and Physical function measures

BMD was measured by dual energy X-ray absorptiometry (DXA) (Lunar iDXA™, UK) performed at the hip, femoral neck and the vertebral column by a fully trained operator according to ionizing radiation medical exposure regulations (IRMER) and scans were subsequently interpreted with the assistance of a radiographer. Results were expressed as grams of BMD per square centimeter (g/cm^2) and as T-scores using the manufacturer's reference database. Osteopenia was defined as a BMD T-score between -1.0 and -2.5 at any site and

osteoporosis defined as a BMD T score > -2.5 at any site (below the young adult mean) [24]. Physical function was primarily assessed by use of the Timed Up and Go test (TUG) which measured the time it took a participant to rise from a chair, walk three meters, turn around, walk back to the chair and sit down again. A score of 12 seconds or more has been reported as an indication of reduced mobility [25]. Additional functionality measures included the PSM and the Instrumental Activities of Daily living scale (IADL) [26].

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (Version 23.0; SPSS UK Ltd; Chersey, UK). Data were assessed for normality and where necessary, data were log-transformed for normalization purposes. Data within tables are primarily expressed as adjusted means with 95% confidence intervals. Estimated marginal means were adjusted for age, gender, BMI, GFR, calcium and vitamin D supplement usage (yes/no). Where appropriate, an independent Student's T-test, one-way ANOVA or ANCOVA with pair-wise comparisons were applied to determine statistical differences between groups (P<0.05). Data were corrected for multiple comparison using the Bonferroni correction. Categorical variables were assessed by chi-square analysis. Hierarchical multiple regression models with adjustment for age, gender, education, BMI, smoking status, alcohol consumption, physical activity (in past two weeks), vitamin D and calcium supplement usage, 25(OH)D concentration, daily milk, yogurt, cheese, total meat (red meat and poultry), total fish (oily and white) and daily egg servings were applied to determine significant predictors of BMD concentrations and physical function measure scores. To determine the predictors of bone health (osteopenia or osteoporosis), a multinomial logistic regression model was used (with normal bone health as the reference category) with relevant co-predictors including the nominal variables: age, BMI, education, 25(OH)D concentration, PTH concentration, GFR, frequency

of daily servings of milk, yogurt, cheese, total meat, total fish and eggs and the categorical variables: gender (reference male), vitamin D supplement user (reference non-vitamin D supplement user), non-smoker (reference smoker), non-alcohol consumer (reference alcohol consumer), physical activity: yes (reference physical activity: no).

Results

General characteristics of participants in the TUDA cohort as defined by gender are shown in Table 1. The majority of participants were female (67.4%), who were significantly older (P=0.004), lighter (P<0.0001), and contained a higher proportion of individuals receiving vitamin D or calcium supplements (P<0.0001) in comparison with males. A higher percentage of females were yogurt consumers with mean daily yogurt servings significantly higher than males (0.42/d vs. 0.32/d respectively) (P<0.0001). In yogurt consumers, the proportion who answered yes to physical activity was 80.9% while in non-consumers it was 74.7%. In participants who had measures of BMD performed, 41.3% had osteopenia while 27% had osteoporosis which was more common in females than males (35.6% vs 14.8% respectively; P<0.0001). Data for BMD, the bone biomarker concentrations and physical function measures across the frequency of daily yogurt intakes (split by gender) are presented in Table 2 and Table 3. In females, after adjustment for covariates (and exclusion of those receiving medications that may affect BMD), total hip BMD was 3.1% higher (P=0.005) and femoral neck BMD was 3.9% higher (P<0.0001) in the high yogurt consumers (>once per day serving) compared to the non-consumers (<once per week serving/never). In males, vertebral BMD was 4.1% higher in low yogurt consumers compared with non-consumers (P=0.028). Similarly, mean vitamin D concentrations (after exclusion of those receiving vitamin D supplements) were 12.9% higher (P=0.006) and mean TRAP 5b concentrations were 9.5% lower (P=0.003) in the male high yogurt consumers compared to the non-consumers. No significant change in concentration

across yogurt consumption was observed for PTH or the remaining bone biomarkers in either gender.

For physical function measures in females, non-consumers of yogurt were 0.9 seconds (6.7%) slower than the high consumers (13.8 vs 12.9 seconds; P=0.020). Similarly, PSM and IADL scores were significantly higher in the yogurt high consumers compared to the non-consumers (P=0.010 & P=0.003 respectively). No significant difference was observed for males. This analysis was then repeated to examine BMD, bone biomarker and physical function measures across frequency of milk and cheese intakes (Supplemental Tables 2-5). No significant difference was observed across milk intake frequencies for BMD or bone biomarker concentrations. However, TUG scores were significantly lower in the non-milk consumers compared to the high milk consumers in both men and women (P<0.05). In addition there were slight increases in PSM and IADL scores across milk intakes in both genders while no significant difference was observed across cheese intake frequencies for any of the measures.

In a hierarchical multiple regression model (Table 3) examining predictors of BMD, bone markers and physical function measures, increasing yogurt intake was a significant positive predictor for BMD in females at all three sites after adjustment for relevant covariates. For instance, with each unit increase in yogurt intake (i.e., an increase of one serving per week) total hip BMD increased by 0.015 g/cm^2 (P=0.002), vertebral BMD by 0.026 g/cm^2 (P=0.005) and femoral neck BMD increased by 0.023 g/cm^2 (P<0.0001). Furthermore, with each yogurt unit increase, TUG scores decreased by 0.59 seconds (P=0.021). In men only, with each unit increase in yogurt intake, concentrations of TRAP 5b decreased by 0.118 µg/L (P<0.0001). Significant predictors of bone health status are outlined in Table 4. Daily yogurt intake was a significant predictor of bone health with each unit increase in yogurt intake associated with a

31% lower risk of having osteopenia (OR 0.69; 95% CI 0.49 – 0.96; *P*=0.032) and a 39% lower risk of being characterized as osteoporotic (OR 0.61; 95% CI 0.42 - 0.89; *P*=0.012) in females and in males a 52% lower risk of osteoporosis (OR 0.48; 95% CI 0.24 - 0.96; *P*=0.038). For females, those on vitamin D supplements had a significantly reduced risk of osteopenia (OR 0.51; 95% CI 0.34 – 0.76; *P*=0.001) and a significantly reduced risk of osteoporosis (OR 0.41; 95% CI 0.26 – 0.64; *P*<0.0001). For males, those on vitamin D supplements also had a significantly reduced risk of osteoporosis (OR 0.40; 95% CI 0.22 – 0.72; *P*=0.003).

Discussion

In this study we observed significant positive associations of increased frequency of yogurt intakes with bone health and measures of physical function in a cohort of older adults. Females with the highest yogurt intakes had significantly higher BMD and better physical function scores compared to individuals with the lowest intakes. Furthermore, we show for the first time that, after adjustment for covariate predictors, each unit increase in yogurt intake significantly decreased the odds of being characterized as osteopenic or osteoporotic in women and as osteoporotic in men.

The significant positive associations of yogurt with BMD within this large study are consistent with previous observations from the Framingham Offspring observational study [17] In 2,733 adults (26-85 yrs), higher yogurt intake was positively associated with trochanteric BMD over a 12-year follow-up with a weak protective trend of yogurt (but not other dairies) on the risk of hip fracture. In a cohort of 61,000 Swedish women (aged 39-74 yrs), fermented milk products (yogurt) were associated with a significant decrease in fracture incidence and mortality over a mean follow-up of 20 years. With each increase in fermented dairy intakes, hip fractures were reduced by 10-15% [18]. Although the current data-set did not have data on

fracture incidence, the effect of increased yogurt intake seen in this cohort has the potential to reduce non-vertebral fractures by up to 46% in women, as fracture risk reduction has been modelled as 46% decrease for 3% hip BMD increase [27]. However it is important to note that the meta-analysis [27] was conducted in women all diagnosed with osteoporosis whereas in the current study only 60% of the women were osteoporotic and thus the potential for yogurt to reduce fractures at the same rate should be viewed with caution. The potential protective effects of yogurt on bone health are also supported by the positive associations of yogurt with the bone biomarker Trap 5b, the concentrations of which were 9.5% lower in those with the highest yogurt intake compared to the lowest, though only in men. Trap 5b is a direct marker of osteoclast number and bone resorption (indicating positive bone balance), with better sensitivity than CTX (a by-product of collagen breakdown) [28] and has been described as one of the most sensitive markers to monitor the response of diet intervention on bone resorption [29]. Significantly fewer men than women were high yogurt consumers, and it is possible this marker was detecting subtle bone turnover changes in men only as not enough yogurt was being consumed to affect BMD but enough to affect bone turnover, though this hypothesis needs to be tested. If the results from the current study are confirmed, there is the potential that increased yogurt intakes may add an inexpensive and relatively low-risk strategy to improve bone health in conjunction with bone treatment. However, future research and randomized controlled trials are needed to explore this approach.

Notably, this study also observed that greater consumption of yogurt was associated with a significantly lower TUG score (6.7% difference lowest vs highest yogurt intakes) in women only. TUG has been described as a composite measure of functional mobility with worse scores associated with poorer muscle strength and balance, both of which are risk factors for falling in older adults [30]. Our results are in agreement with Lana et al. who observed that higher

consumption of yogurt (and milk) was associated with a lower risk of frailty and a lower risk of a slow walking speed in 1,871 community dwelling older adults [31]. Furthermore, in a cross-sectional study of elderly Australian women (n 1,456), higher dairy intake was associated with increased grip strength and decreased likelihood of a lower TUG score [32].

A number of potential mechanisms may explain the observed positive associations. Yogurt naturally contains significant concentrations of bone promoting minerals and vitamins [10-14] which have also been associated with improved frailty measures [33,34]. In data from the Framingham Heart Study offspring cohort, yogurt consumers were 47% and 55% less likely to have inadequate intakes of vitamins B2 and B12 (respectively) [35], while in 2,797 Italian adults (aged 18-97 yrs), yogurt consumers were more likely to have adequate intakes of vitamins and minerals compared to non-consumers [36]. Yogurt also contains significant quantities of protein, bio-active peptides and bio-cultures which have been associated with bone health and immunological benefits [37-43]. For example, yogurt (and other dairy products) contain branched chain amino acids (BCAAs) which are potent stimulators of muscle protein synthesis [44,45]. Furthermore, in a recent review it was suggested that the modifiable nature of the gut microbiome could provide a potential therapeutic target to intervene in musculoskeletal conditions of aging [46]. It is perhaps this unique combination of macro and micronutrients with bio-active compounds within yogurt that confers bone promotion and improved physical function. It is also possible that increased yogurt intakes could also be a reflection of a long term dietary habit of an overall healthy eating pattern and lifestyle [36], though diet quality (including vitamin D and calcium) was adjusted for in the current analysis. Yogurt has been the target of some criticism, especially with the renewed concerns regarding excess sugar intakes and associations with obesity [47] given that some processed ‘sweetened’ yogurts can contain substantial quantities of sugar [48]. Yet not all yogurts have a high sugar

content [49] and further exploration is required to identify the types of yogurts and the individual components within that may exhibit health benefits.

We also examined the associations of the other dairy products (milk and cheese) with BMD and functionality. We observed no significant difference in BMD across milk intakes, in line with inconsistent data from previous observational studies. For example, some studies have observed strong associations between childhood and adolescent milk consumption with BMD [50]. For older adults (>60 yrs), studies have observed no associations or a negative association of milk intakes with fracture risk [50]. The majority of positive randomized trials with milk which have observed significant decreases in the concentrations of bone biomarkers and improvements in bone metabolism have all utilized fortified milk [51]. The milk intakes in the current study were not heavily fortified at this time period (2008-2012) and could account for the lack of any such association. Furthermore, we have previously reported that in this population, with increasing age, milk intakes increased while yogurt intakes significantly decreased [22]. This could help explain why some of the physical function measures became poorer with increased milk intakes. As milk intakes increased, we suggest that there was a loss of a particular protective component within the yogurt that enhanced bone health/improved physical function, though this hypothesis requires verification. Interestingly, we observed no significant associations of cheese intakes with BMD though male participants with osteoporosis were more likely to have a higher frequency of cheese consumption. Cheese products generally have a different nutritional profile in comparison with yogurts and we previously observed in TUDA that cheese intakes had no significant effect on the concentrations of vitamin D, folate, vitamin's B12, B6 or B2 [22]. Furthermore, it has been suggested that the high sodium content of certain cheeses could be less beneficial for bone health by negatively altering calcium metabolism, though few studies have examined this issue

[52] and the sodium-induced calciuria hypothesis has not been supported by any long term observations. Moreover, fortified cheese products have been positively associated with bone metabolism [53]. Further research is needed to identify the relationship between cheese intakes and BMD in men.

Our study has several limitations. The data are observational and cross-sectional, and such observed associations between yogurt intakes and bone health do not necessarily indicate a causal relationship. However, one of the major strengths of this study was the size, as to the best of our knowledge it is the largest observational study conducted to date investigating such associations. Potential weaknesses of this study also include our reliance on self-reported intakes and we were unable to quantify the dairy or yogurt intakes through food dairies or other more quantitative dietary collection (and thus did not have information on serving sizes or product types). However, although we could not adjust for total energy intake, we did adjust for frequency of intake of other important dietary components including meat, fish, egg and other dairy constituents which can give a proxy measure of diet quality. Furthermore, those with severe cognitive impairment or frailty were removed from the analysis to increase recall accuracy.

In conclusion, to our knowledge, this is the largest study to demonstrate an association between the frequency of yogurt intakes, BMD, bone biomarkers and measures of physical function exclusively within free-living, older adults (>60y). The findings provide evidence that lower frequency of yogurt intake is significantly associated with a lower BMD and that improving yogurt intakes could be a valuable and cost-effective health measure for maintaining bone health and in reducing frailty in older adults. Future RCT trials are required to assess and investigate the efficacy of such approaches.

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Table 1. Demographic and health characteristics of the TUDA cohort study by gender[a]

Variable	Total (*n*= 4,310)	Male (*n*= 1,405)	Female (*n*= 2,905)	*P* value
Age,[b] y	73.1 (7.9)	72.6 (7.8)	73.3 (8.0)	0.004
60-69,[c] y *n* (%)	1690 (39.2)	567 (40.4)	1123 (38.7)	0.284
70-79,[c] y *n* (%)	1697 (39.4)	568 (40.4)	1129 (38.9)	0.325
>80,[c] *n* (%)	923 (21.4)	270 (19.2)	653 (22.5)	0.014
Age Finished Education,[b] (yrs)	16.2 (3.0)	16.2 (3.2)	16.2 (2.9)	0.564
Health & Lifestyle				
BMI,[b] kg/m^2	28.0 (5.3)	28.6 (4.4)	27.7 (5.7)	<0.0001
GFR,[b] ml/min	69.4 (24.4)	77.1 (25.4)	65.6 (22.9)	<0.0001
Current smoker,[c] *n* (%)	515 (12.0)	156 (11.1)	359 (12.4)	0.231
Current alcohol consumer,[c] *n* (%)	2551 (59.2)	919 (65.5)	1632 (56.2)	<0.0001
Physical activity in last two weeks,[c] *n* (%)	3403 (79.0)	1094 (77.9)	2309 (79.5)	0.214
Receives Bone medications,[c] *n* (%)	1484 (34.4)	244 (17.4)	1240 (42.7)	<0.0001
Yogurt consumer,[c] *n* (%)	2658 (61.7)	725 (51.6)	1933 (66.5)	<0.0001
Milk (as a drink) consumer,[c] *n* (%)	1806 (42.9)	640 (46.4)	1166 (41.1)	0.001
Cheese consumer,[c] *n* (%)	3651 (84.7)	1209 (86.0)	2442 (84.1)	0.089
Supplement use,[c] *n* (%)				
Vitamin D supplement user	2042 (47.8)	447 (33.2)	1595 (58.4)	<0.0001
Calcium supplement user	1742 (40.4)	308 (21.9)	434 (49.4)	<0.0001
Phosphate supplement user	262 (6.1)	82 (5.8)	180 (6.2)	0.643

[a] Values are means (±SD) for continuous variables.

[b] Student's independent *t* test was used to test differences between log-transformed continuous variables.

[c] Chi-square tests were used to test differences between categorical variables.

Table 2. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily yogurt intake in females in the TUDA cohort study[a]

	Tertile of daily yogurt intakes								
	Non-consumer			Low consumer			High consumer		
	Mean yogurt frequency (0.0 daily / <once per week/never) *n*= 970			Mean yogurt frequency (0.34 daily / 2-3 times per week) *n*= 826			Mean yogurt frequency (1.03 daily / >once per day) *n*= 1109		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	320	0.890	0.877 – 0.903	331	0.904	0.891 – 0.917	406	0.918*	0.907 – 0.930
Femoral Neck	319	0.824	0.812 – 0.836	331	0.843	0.831 – 0.855	405	0.857**	0.846 – 0.867
Vertebral	260	1.005	0.984 – 1.025	251	1.027	1.006 – 1.047	330	1.036	1.018 – 1.054
Bone health biomarkers									
CTX,[b] ng/ml	226	0.34	0.32 – 0.36	258	0.33	0.31 – 0.35	278	0.32	0.30 – 0.34
OC,[b] ng/ml	225	19.2	18.0 – 20.5	252	18.7	17.5 – 19.8	278	18.8	17.7 – 19.9
BAP,[b] µg/L	226	17.8	16.9 – 18.7	252	18.0	17.1 – 18.8	278	17.7	16.9 – 18.5
TRAP 5b,[b] µg/L	226	3.30	3.17 – 3.43	252	3.29	3.17 – 3.41	279	3.24	3.12 – 3.35
25(OH)D,[c] nmol/L	412	41.0	38.9 – 43.2	347	43.4	41.1 – 45.7	361	43.5	41.2 – 45.8
PTH,[b] pg/ml	505	50.0	47.5 – 52.5	446	47.0	44.4 – 49.7	578	46.3	43.9 – 48.6
Physical function measures[c]									
Timed up and Go, (sec)	868	13.8	13.3 – 14.2	740	13.0	12.5 – 13.5	1,016	12.9*	12.5 – 13.3
IADL	906	24.4	24.2 – 24.6	751	24.5	24.3 – 24.8	1,014	24.8*	24.6 – 25.0
PSM	913	22.9	22.8 – 23.0	767	22.9	22.8 – 23.0	1,035	23.1*	23.0 – 23.2

[a] Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. *,**Different from the lowest yogurt intake tertile: *P<0.05, **P<0.0001. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 3-4 times per week); high consumer frequency range (>0.50 – 2.00 units / >3-4 times per week to twice per day).

[b]Adjusted for age, education, BMI, GFR, physical activity, total daily serving milk (glass only), total daily serving of cheese, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjusted for age, BMI, total daily serving milk (glass only), total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.

Table 3. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily yogurt intake in males in the TUDA cohort study[a]

	Tertile of daily yogurt intakes								
	Non-consumer			Low consumer			High consumer		
	Mean yogurt frequency (0.0 daily / <once per week/never) *n*= 680			Mean yogurt frequency (0.29 daily / 2-3 times per week) *n*= 392			Mean yogurt frequency (1.00 daily / >once per day) *n*= 333		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	339	1.041	1.026 – 1.056	239	1.057	1.039 – 1.075	185	1.058	1.038 – 1.079
Femoral Neck	339	0.925	0.911 – 0.939	239	0.945	0.928 – 0.962	185	0.947	0.928 – 0.966
Vertebral	271	1.207	1.183 – 1.231	180	1.258*	1.229 – 1.287	134	1.235	1.201 – 1.269
Bone health biomarkers									
CTX,[b] ng/ml	306	0.27	0.25 – 0.29	231	0.27	0.25 – 0.29	169	0.27	0.25 – 0.29
OC,[b] ng/ml	305	14.7	13.9 – 15.7	231	14.9	13.9 – 15.8	169	15.0	13.8 – 16.2
BAP,[b] µg/L	305	16.2	15.3 – 17.1	231	15.7	14.6 – 16.7	168	16.2	15.0 – 17.5
TRAP 5b,[b] µg/L	306	2.96	2.87 – 3.06	231	2.89	2.78 – 3.00	169	2.69**	2.56 – 2.82
25(OH)D,[c] nmol/L	443	41.8	39.7 – 44.0	243	49.3**	46.3 – 52.2	205	47.6*	44.4 – 50.8
PTH,[b] pg/ml	527	45.2	42.7 – 47.8	310	43.7	40.4 – 47.0	258	47.9	44.3 – 51.5
Physical function measures[c]									
Timed up and Go, (sec)	627	13.3	12.6 – 13.9	361	12.0*	11.1 – 12.8	302	12.7	11.8 – 13.6
IADL	636	24.6	24.3 – 24.9	372	25.4*	25.0 – 25.8	309	24.7	24.4 – 25.2
PSM	647	23.2	23.1 – 23.4	374	23.4	23.2 – 23.5	315	23.2	23.0 – 23.4

[a] Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. *,**Different from the lowest yogurt intake tertile: *P<0.05, **P<0.0001. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 3-4 times per week); high consumer frequency range (>0.50 – 2.00 units / >3-4 times per week to twice per day).

[b]Adjusted for age, education, BMI, GFR, physical activity, total daily serving milk (glass only), total daily serving of cheese, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjusted for age, BMI, total daily serving milk (glass only), total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.

Table 4. Yogurt consumption as a predictor of markers of bone health and physical function in the TUDA cohort study[a]

	Total Hip BMD,[b] g/cm^2	Femoral Neck BMD,[b] g/cm^2	Vertebral BMD,[b] g/cm^2	TRAP 5b,[b] µg/L	Timed up and go,[c] sec	IADL[c]	PSM[c]
Variable	**β**	**β**	**β**	**β**	**β**	**β**	**β**
Total sample	0.015 (0.007)	0.023 (0.006)	0.026 (0.011)	-0.118 (0.055)	-0.599 (0.252)	0.220 (0.115)	0.084 (0.054)
P-value	0.015	<0.0001	0.016	0.032	0.018	0.056	0.121
Female only	0.024 (-0.008)	0.031 (0.007)	0.034 (0.012)	0.015 (-0.077)	-0.641 (0.277)	0.272 (0.126)	0.157 (0.065)
P-value	0.002	<0.0001	0.005	0.847	0.021	0.031	0.016
Male only	0.004 (-0.013)	0.009 (-0.012)	0.012 (-0.021)	-0.292 (0.080)	-0.496 (-0.543)	0.06 (-0.248)	-0.141 (-0.096)
P-value	0.761	0.431	0.557	<0.0001	0.361	0.809	0.144

[a]Values are unstandardized Beta (β) coefficients (standard error) derived from a hierarchical multiple regression analysis.

[b]Adjustment for age, gender (total sample only), education, BMI, smoking status, alcohol consumption, vitamin D or calcium supplement use, 25(OH)D, GFR, physical activity, total daily serving milk (glass only), total daily serving of cheese, total daily serving of meat (red meat and poultry), total daily serving of fish (white and oily) and total daily serving of eggs. (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjustment for age, gender (total sample only), BMI, total daily serving milk (glass only), total daily serving of cheese, total daily serving of meat (red meat and poultry), total daily serving of fish (white and oily) and total daily serving of eggs.

Abbreviations: IADL, Instrumental Activities of Daily Living Scale; PSM, Physical Self-Maintenance Scale; TRAP 5b, Tartrate-resistant acid phosphatase 5b; 25(OH)D, 25-hydroxyvitamin D.

Table 5. The predictors of bone health status of females within the TUDA cohort study[a]

Variable	Odds ratio [95% CI]	*P* Value	Odds ratio [95% CI]	*P* Value
	Osteopenia vs Normal		*Osteoporosis vs Normal*	
	(*n* = 411 vs 266)		(*n* = 360 vs 266)	
Age (y)	1.02 [0.99-1.06]	0.073	1.04 [1.00-1.08]	0.018
BMI (kg/m^2)	0.93 [0.89-0.96]	<0.0001	0.79 [0.76-0.83]	<0.0001
25(OH)D (nmol/L)	0.99 [0.99-1.00]	0.869	1.00 [0.99-1.01]	0.148
Vitamin D supplement user	0.51 [0.34-0.76]	0.001	0.41 [0.26-0.64]	<0.0001
PTH (pg/mL)	1.00 [0.99-1.01]	0.322	1.00 [0.99-1.01]	0.068
Education (yrs)	0.97 [0.91-1.03]	0.360	0.95 [0.88-1.02]	0.181
Non-Smoker[b]	0.89 [0.50-1.58]	0.710	0.61 [0.34-1.12]	0.116
Non-Alcohol user[c]	0.85 [0.60-1.20]	0.372	1.06 [0.72-1.57]	0.740
Physical activity: Yes[d]	0.89 [0.56-1.43]	0.645	0.61 [0.35-1.56]	0.078
GFR (ml/min)	0.99 [0.98-1.00]	0.810	1.00 [0.99-1.01]	0.601
Daily yogurt serving	0.69 [0.49-0.96]	0.032	0.61 [0.42-0.89]	0.012
Daily milk serving	0.77 [0.53-1.11]	0.167	0.75 [0.49-1.13]	0.175
Daily cheese serving	1.05 [0.65-1.69]	0.831	1.18 [0.70-2.00]	0.516
Daily meat serving	0.91 [0.58-1.41]	0.676	1.16 [0.70-1.92]	0.546
Daily fish serving	0.94 [0.39-2.26]	0.892	0.48 [0.17-1.35]	0.168
Daily egg serving	1.24 [0.63-2.46]	0.528	1.66 [0.76-3.61]	0.201

[a]Values are odds ratios (95 % CI lower and upper) derived from a multi-nominal logistic regression analysis. Overall reference category is normal bone health based on the WHO definition of osteopenia and osteoporosis [24] using the combination of total hip, femoral neck or vertebral BMD where available. Participants receiving medications that could affect bone metabolism were removed from the analysis.

[b]Reference is smoker

[c]Reference is user.

[d]Reference is physical activity: No in the last two weeks

Table 6. The predictors of bone health status of males within the TUDA cohort study[1]

Variable	Odds ratio [95% CI]	*P* Value	Odds ratio [95% CI]	*P* Value
	Osteopenia vs Normal		*Osteoporosis vs Normal*	
	(*n* = 332 vs 315)		(*n* = 104 vs 315)	
Age (y)	0.98 [0.95-1.01]	0.318	1.01 [0.96-1.06]	0.643
BMI (kg/m^2)	0.96 [0.91-1.01]	0.128	0.80 [0.73-0.87]	<0.0001
25(OH)D (nmol/L)	1.00 [0.99-1.00]	0.703	0.99 [0.98-1.00]	0.213
Vitamin D supplement user	0.79 [0.52-1.21]	0.286	0.40 [0.22-0.72]	0.003
PTH (pg/mL)	1.00 [0.99-1.01]	0.238	1.01 [1.00-1.01]	0.026
Education (yrs)	0.95 [0.91-1.00]	0.106	0.96 [0.88-1.04]	0.389
Non-Smoker[3]	0.58 [0.32-1.07]	0.085	0.39 [0.18-0.85]	0.019
Non-Alcohol user[2]	0.77 [0.53-1.11]	0.170	0.94 [0.54-1.64]	0.846
Physical activity: Yes[4]	0.59 [0.37-0.92]	0.022	1.09 [0.51-2.35]	0.812
GFR (ml/min)	0.98 [0.97-0.99]	0.016	0.98 [0.97-1.00]	0.128
Daily yogurt serving	0.88 [0.59-1.30]	0.537	0.48 [0.24-0.96]	0.038
Daily milk serving	0.84 [0.58-1.19]	0.338	1.58 [0.99-2.52]	0.055
Daily cheese serving	1.79 [1.11-2.88]	0.016	2.36 [1.16-4.82]	0.018
Daily meat serving	0.64 [0.42-0.99]	0.045	1.10 [0.58-2.07]	0.756
Daily fish serving	0.74 [0.30-1.81]	0.522	0.69 [0.17-2.85]	0.617
Daily egg serving	1.27 [0.69-2.32]	0.439	1.16 [0.47-2.86]	0.740

[1]Values are odds ratios (95 % CI lower and upper) derived from a multi-nominal logistic regression analysis. Overall reference category is normal bone health based on the WHO definition of osteopenia and osteoporosis [24] using the combination of total hip, femoral neck or vertebral BMD where available. Participants receiving medications that could affect bone metabolism were removed from the analysis.

[2]Reference is user.

[3]Reference is smoker.

[4]Reference is physical activity: No in the last two weeks

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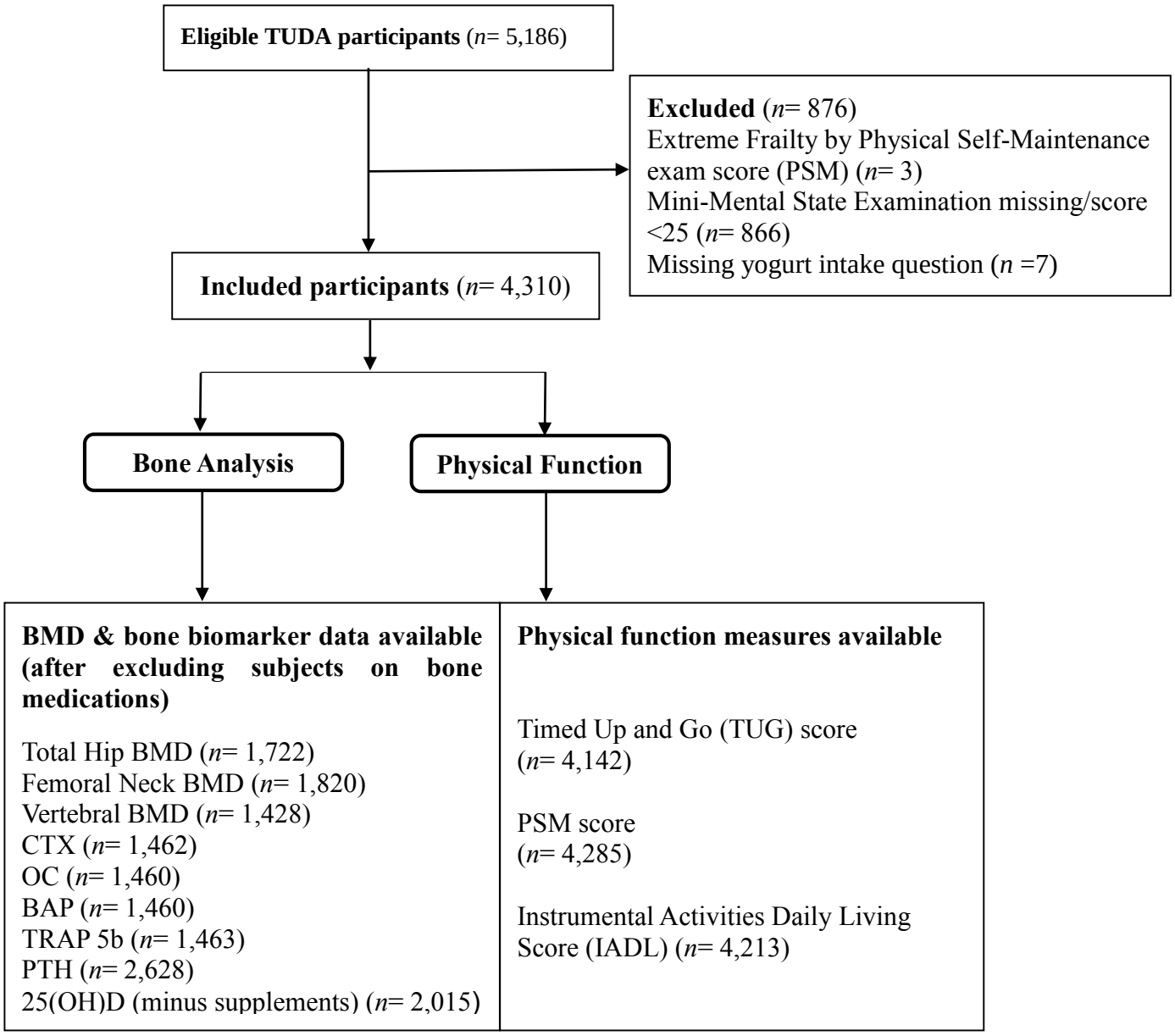

Supplemental Figure 1. Study Design for yogurt intake frequency analysis within the TUDA cohort study

Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; OC, Osteocalcin; Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; 25(OH)D, 25-hydroxyvitamin D.

Supplemental Table 1. Calculation of daily yogurt and dairy intakes in the TUDA cohort study[1]

Questionnaire Dairy Intake Frequency	Daily Yogurt intake frequency	Daily Milk intake frequency	Daily Cheese intake frequency
Twice per day	2	2	2
Once per day	1	1	1
5-6 times per week	0.785	0.785	0.785
3-4 times per week	0.5	0.5	0.5
1-2 times per week	0.21	0.21	0.21
<Once per week	0.07	0.07	0.07

[1]Values are self-reported intakes derived from a modified food frequency questionnaire (FFQ)

Supplemental Table 2. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily milk intake in females in the TUDA cohort study[a]

	Tertile of daily milk intakes								
	Non-consumer			Low consumer			High consumer		
	Mean milk frequency (0.0 daily / <once per week/never) *n*= 1,746			Mean milk frequency (0.18 daily / 1-2 times per week) *n*= 305			Mean milk frequency (1.00 daily / >once per day) *n*= 854		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	673	0.907	0.898 – 0.916	133	0.903	0.883 – 0.923	252	0.902	0.888 – 0.917
Femoral Neck	672	0.845	0.837 – 0.853	133	0.838	0.820 – 0.857	250	0.838	0.824 – 0.852
Vertebral	545	1.029	1.015 – 1.043	99	1.026	0.993 – 1.059	197	1.008	0.985 – 1.032
Bone health biomarkers									
CTX,[b] ng/ml	465	0.33	0.32 – 0.35	107	0.34	0.31 – 0.37	184	0.3`	0.29 – 0.34
OC,[b] ng/ml	464	19.2	18.3 – 20.0	107	18.5	16.7 – 20.2	184	18.4	17.1 – 19.8
BAP,[b] µg/L	466	17.8	17.1 – 18.4	107	17.3	16.0 – 18.6	183	18.3	17.3 – 19.3
TRAP 5b,[b] µg/L	566	3.31	3.22 – 3.40	107	3.22	3.04 – 3.41	184	3.21	3.07 – 3.35
25(OH)D,[c] nmol/L	691	43.2	41.5 – 44.8	129	41.8	38.0 – 45.7	301	41.5	39.0 – 44.0
PTH,[b] pg/ml	942	46.7	44.9 – 48.6	167	49.0	44.6 – 53.3	420	49.4	46.7 – 52.1
Physical function measures[c]									
Timed up and Go, (sec)	1,688	12.8	12.5 – 13.1	295	12.5	11.8 – 13.3	808	13.7*	13.2 – 14.2
IADL	1,706	24.9	24.7 – 25.0	298	24.8	24.5 – 25.2	831	24.2*	24.0 – 24.4
PSM	1,738	23.1	23.0 – 23.2	302	22.9	22.8 – 23.1	847	22.9*	22.8 – 23.0

[a]Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. *,**Different from the lowest milk intake tertile: **P*<0.05, ***P*<0.0001. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 1-2 times per week); high consumer frequency range (0.50 – 2.00 units / >3-4 times per week to twice per day).

[b]Adjusted for age, education, BMI, GFR, physical activity, total daily serving of yogurt, total daily serving of cheese, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjusted for age, BMI, total daily serving yogurt, total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.

Supplemental Table 3. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily cheese intake in females in the TUDA cohort study[a]

	Tertile of daily cheese intakes								
	Non-consumer			Low consumer			High consumer		
	Mean cheese frequency (0.0 daily / <once per week/never) *n*= 602			Mean cheese frequency (0.33 daily / 2-3 times per week) *n*= 1,673			Mean cheese frequency (0.95 daily / >once per day) *n*= 631		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	241	0.903	0.888 – 0.918	614	0.908	0.899 – 0.918	202	0.898	0.882 – 0.914
Femoral Neck	241	0.840	0.826 – 0.854	612	0.846	0.838 – 0.855	202	0.833	0.818 – 0.849
Vertebral	187	1.015	0.991 – 1.039	489	1.026	1.011 – 1.041	165	1.026	1.001 – 1.052
Bone health biomarkers									
CTX,[b] ng/ml	192	0.32	0.30 – 0.34	434	0.33	0.31 – 0.35	130	0.34	0.31 – 0.37
OC,[b] ng/ml	192	19.0	17.7 – 20.4	438	18.7	17.8 – 19.6	130	19.4	17.8 – 21.0
BAP,[b] µg/L	192	17.9	16.9 – 18.8	434	18.0	17.3 – 18.6	130	17.2	16.0 – 18.4
TRAP 5b,[b] µg/L	192	3.25	3.11 – 3.39	435	3.28	3.19 – 3.37	130	3.29	3.13 – 3.46
25(OH)D,[c] nmol/L	221	40.6	37.7 – 43.5	532	43.4	41.5 – 45.3	163	39.9	36.5 – 43.3
PTH,[b] pg/ml	335	47.0	44.0 – 50.1	893	46.9	45.1 – 48.8	301	50.7	47.5 – 54.0
Physical function measures[c]									
Timed up and Go, (sec)	577	13.3	12.8 – 13.9	1,603	12.9	12.6 – 13.2	611	13.2	12.6 – 13.7
IADL	583	24.5	24.3 – 24.7	1,634	24.8	24.6 – 24.9	618	24.6	24.4 – 24.9
PSM	596	23.0	22.9 – 23.2	1,662	23.0	23.0 – 23.1	629	23.0	22.9 – 23.1

[1]Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. No significant difference observed from the lowest cheese intake tertile to the highest. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 3-4 times per week); high consumer frequency range (>0.50 – 2.00 units / >3-4 times per week to twice per day).

[2]Adjusted for age, education, BMI, GFR, physical activity, total daily serving milk (glass only), total daily serving of yogurt, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[3]Adjusted for age, BMI, total daily serving milk (glass only), total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.

Supplemental Table 4. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily cheese intake in males in the TUDA cohort study[a]

	Tertile of daily cheese intakes								
	Non-consumer			Low consumer			High consumer		
	Mean cheese frequency (0.0 daily / <once per week/never) *n*= 269			Mean cheese frequency (0.34 daily / 2-3 times per week) *n*= 835			Mean cheese frequency (0.95 daily / >once per day) *n*= 304		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	176	1.064	1.043 – 1.088	447	1.048	1.035 – 1.061	140	1.041	1.048 – 1.064
Femoral Neck	176	0.953	0.934 – 0.972	447	0.932	0.920 – 0.944	140	0.929	0.907 – 0.951
Vertebral	129	1.246	1.211 – 1.280	342	1.221	1.200 – 1.242	114	1.234	1.197 – 1.271
Bone health biomarkers									
CTX,[b] ng/ml	173	0.27	0.25 – 0.30	413	0.27	0.25 – 0.28	120	0.27	0.24 – 0.30
OC,[b] ng/ml	173	15.1	13.9 – 16.3	413	15.0	14.3 – 15.8	121	13.8	12.4 – 15.2
BAP,[b] µg/L	172	16.4	15.2 – 17.6	412	15.8	15.0 – 16.6	120	16.3	14.8 – 17.7
TRAP 5b,[b] µg/L	173	2.94	2.81 – 3.06	413	2.86	2.78 – 2.95	120	2.81	2.66 – 2.97
25(OH)D,[c] nmol/L	163	45.7	42.0 – 49.3	473	45.1	43.0 – 47.2	172	46.3	42.8 – 49.3
PTH,[b] pg/ml	221	44.0	40.0 – 47.9	647	46.3	44.0 – 48.6	227	44.2	40.4 – 48.1
Physical function measures[c]									
Timed up and Go, (sec)	255	12.1	11.1 – 13.1	804	12.8	12.2 – 13.3	288	13.2	12.3 – 14.2
IADL	261	25.1	24.6 – 25.5	815	25.0	24.7 – 25.2	298	24.8	24.3 – 25.2
PSM	265	23.4	23.2 – 23.6	828	23.2	23.1 – 23.3	301	23.3	23.1 – 23.5

[a]Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. No significant difference observed from the lowest cheese intake tertile to the highest. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 3-4 times per week); high consumer frequency range (>0.50 – 2.00 units / >3-4 times per week to twice per day).

[b]Adjusted for age, education, BMI, GFR, physical activity, total daily serving milk (glass only), total daily serving of yogurt, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjusted for age, BMI, total daily serving milk (glass only), total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.

Supplemental Table 5. Comparison of bone mineral density (BMD), biomarkers of bone health and mean frailty measures across frequencies of daily milk intake in males in the TUDA cohort study[a]

	Tertile of daily milk intakes								
	Non-consumer			Low consumer			High consumer		
	Mean milk frequency (0.0 daily / <once per week/never) *n*= 768			Mean milk frequency (0.18 daily / 2-3 times per week) *n*= 201			Mean milk frequency (0.99 daily / >once per day) *n*= 438		
Variable	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
BMD region,[b] g/cm^2									
Total Hip	424	1.050	1.036 – 1.063	132	1.050	1.026 – 1.074	207	1.051	1.032 – 1.071
Femoral Neck	424	0.939	0.926 – 0.951	132	0.936	0.914 – 0.958	207	0.932	0.915 – 0.950
Vertebral	331	1.234	1.213 – 1.256	98	1.216	1.176 – 1.256	156	1.226	1.195 – 1.257
Bone health biomarkers									
CTX,[b] ng/ml	392	0.26	0.25 – 0.28	125	0.27	0.25 – 0.30	189	0.28	0.26 – 0.30
OC,[b] ng/ml	391	15.0	14.3 – 15.8	125	14.3	12.9 – 15.6	189	14.8	13.7 – 15.9
BAP,[b] µg/L	391	16.1	15.2 – 16.9	125	15.9	14.5 – 17.4	188	16.1	14.9 – 17.2
TRAP 5b,[b] µg/L	392	2.85	2.76 – 2.93	125	2.95	2.80 – 3.10	189	2.87	2.75 – 2.99
25(OH)D,[c] nmol/L	486	44.7	42.6 – 46.8	127	46.5	42.4 – 50.6	279	45.5	42.8 – 48.3
PTH,[b] pg/ml	591	45.7	43.3 – 48.1	168	42.0	37.5 – 46.5	336	46.6	43.5 – 49.8
Physical function measures[c]									
Timed up and Go, (sec)	739	12.4	11.8 – 13.0	193	11.9	10.8 – 13.0	415	13.7*	12.9 – 14.5
IADL	753	25.2	25.0 – 25.5	197	25.2	24.7 – 25.7	424	24.3**	24.0 – 24.7
PSM	762	23.3	23.2 – 23.4	200	23.4	23.2 – 23.6	432	23.1*	23.0 – 23.2

[a]Values are estimated marginal means (95% CI) adjusted for multiple covariates. Differences in means were assessed by pair-wise comparisons and adjusted for multiple comparisons: Bonferroni correction. *,**Different from the lowest milk intake tertile: **P*<0.05, ***P*<0.0001. Non-consumer frequency range (0 – 0.07 units / <once per week/never); low consumer frequency range (>0.07- 0.50 units / >once per week to 3-4 times per week); high consumer frequency range (>0.50 – 2.00 units / >3-4 times per week to twice per day).

[b]Adjusted for age, education, BMI, GFR, physical activity, total daily of yogurt, total daily serving of cheese, calcium or vitamin D supplements (Participants receiving medications that could affect bone metabolism were removed from the analysis).

[c]Adjusted for age, BMI, total daily serving milk (glass only), total daily serving of cheese; (participants receiving vitamin D supplements were removed from the 25(OH)D analysis). Abbreviations: BAP, Bone specific alkaline phosphatase; CTX, C-terminal telopeptides of type I collagen; IADL, Instrumental Activities of Daily Living Scale; OC, Osteocalcin; PSM, Physical Self-Maintenance Scale; PTH, Parathyroid hormone; TRAP 5b, Tartrate-resistant acid phosphatase 5b; /d, per day; 25(OH)D, 25-hydroxyvitamin D. OC reference range for males of 9.6-40.8 ng/ml and for postmenopausal women 12.8-55.0 ng/ml; CTX reference range is 0.020 ng/mL to 3.380 ng/ml; BAP reference range

for males of 5.7-32.9 µg/ml and for postmenopausal women 5.5-27.1 µg/ml; TRAP 5b reference range for males of 55-79 ng/ml and for postmenopausal women 41-81 ng/ml; Intact PTH measurement range of 1.2 – 5000 pg/ml. and 25(OH)D detection range 7.5-624 nmol/L.